

**Growth performance, feed utilisation and body composition of  
advanced nursing Nile tilapia (*Oreochromis niloticus*) fed diets  
containing Black Soldier Fly (*Hermetia illucens*) larvae meal**

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## Abstract

A 32-day experiment was conducted to evaluate the effects on the performance, feed utilisation efficiency and body composition of a strategic inclusion of Black Soldier Fly larvae meal (MM) in a commercially formulated diet for advance nursing Nile tilapia (*Oreochromis niloticus*). Four isonitrogenous and isoenergetic diets were commercially formulated and manufactured as a control and 3 test diets with strategic inclusions of MM inclusions (0, 30, 50 and 80 g kg<sup>-1</sup>) and poultry byproduct meal substituting gradually three conventional expensive feedstuffs: fish meal, fish oil and soybean meal. Fish (5.7±0.5 g fish<sup>-1</sup>) were nursed in a cage-in-lake system (Volta Lake, Ghana), under conditions similar to commercial farming practices. Control and experimental diets were fed to triplicate cages by hand to visual satiety, 6 times day<sup>-1</sup>. Growth performance (final weight; weight gain and SGR); feed utilisation efficiency indices (FCR and PER) and feed intake were not significantly different ( $P \geq 0.05$ ) between treatments. Survival was significantly different ( $P < 0.05$ ) but more likely explained by the stress related to frequent handling on the smaller fish. Fish whole body composition (dry matter, crude protein, lipid, ash and fibre) was unaffected by the treatment ( $P \geq 0.05$ ), except for the fatty acid compositions which mirrored that of the diets.

## 38    **Introduction**

39    Farmed fish contribute to food security and represent a rich source of dietary animal  
40    protein, micronutrients and fatty acids (FA) in low-income countries (Beveridge *et al.*  
41    2013). In Ghana, for instance, most aquaculture production (around 80%) consists of Nile  
42    tilapia (*Oreochromis niloticus*) (FAO 2005), but local fish farmers struggle to compete  
43    with cheaper imports from China and are constrained by both availability, quality and  
44    cost of pelleted fish feeds and feed ingredients (Hecht 2007; Rurangwa *et al.* 2015).  
45    Conventional feed ingredients such as fish meal (FM), fish oil (FO) and plants protein  
46    sources (oilseed plants, grain legumes, etc.), for which there is an increasing demand due  
47    to the intensification of farming methods relying on complete fish feeds (Tacon & Metian  
48    2008), are available in low income countries such as Ghana, consisting either of poor  
49    quality local products or high-cost imported items (Gabriel *et al.* 2007; Obirikorang *et al.*  
50    2015). Importance of quality ingredients and artificial feeds, even for herbivorous species  
51    such as tilapia, makes perfect sense at critical stages (juveniles or broodstock) when fish  
52    are maintained under intensive clear-water farming conditions and depend entirely on  
53    nutritionally complete diets (Tacon 1988). Global research for the identification of cost-  
54    effective substitutes to conventional materials continues (El-Sayed & Tacon 1997; El-  
55    Sayed 2004; Karalazos 2007; Hasan *et al.* 2007; Ayoola 2010; Obirikorang *et al.* 2015).  
56    Insect meals such as fly larvae or maggots meals (MM) have been identified as high  
57    protein and valuable feed ingredient for livestock in general (Veldkamp *et al.* 2012; van  
58    Huis *et al.* 2013; Makkar *et al.* 2014) and freshwater fish specifically, given their natural  
59    feeding habits (Bailey & Harrison 1948; Randall 1967; Odesanya *et al.* 2011; Barroso *et*  
60    *al.* 2014; Henry *et al.* 2015). Previous research on tilapia juveniles have shown that both  
61    meals from housefly larvae (*Musca domestica*) and blowfly larvae (*Chrysomya*

*megacephala*) can replace up to 100% of the FM in practical diets for tilapia fingerlings without affecting fish performance compared to FM-based control diets (Ogunji *et al.* 2008a, b, c; Sing *et al.* 2014). On the other hand, fresh Black Soldier Fly (BSF, *Hermetia illucens*) larvae fed whole or chopped to blue tilapia (*Oreochromis aureus*) reduced significantly the fish growth (Bondari & Sheppard 1987). BSF larvae meal has been used as a substitute to FM in several fish species diets except tilapia (Makkar *et al.* 2014; Henry *et al.* 2015). In complete diets, BSF meal, which can be produced locally (Devic *et al.* 2013), may be blended with other good protein sources such as poultry byproduct meal (PBM) to substitute high-priced FM, FO and soybean meal (SBM). This study investigates the effects on the performance, feed utilisation efficiency and body composition of the strategic inclusions BSF larvae meal (MM) in commercially formulated diets for juvenile Nile tilapia (*O. niloticus*).

## **Material and Methods**

### *Diets*

BSF larvae (*H. illucens*) meal (MM) was produced within a pilot system located in Greater Accra (Ghana) described by Charlton *et al.* (2015). Larvae were fed on a substrate mix composed of 35% spent grain (brewery solid waste) or wheat bran (depending on availability), 22% processing wastes from a local fish feed factory, 12% yeast slurry (brewery waste water) and 31% water (bringing the moisture content to approximately 50%) and were harvested after 13 days of development (prior reaching the prepupae stage). Oven-dried larvae (60-80°C, 2 hours) were subsequently ground into a fine and homogeneous meal using a flour mill machine. Nutritional composition of the MM was

analysed (Table 1) according to the methods described below in order to assist in diet formulation.

Raanan Fish Feed West Africa (Prampram Fishfeed Factory, Ghana) supplied the other feed ingredients, formulated and prepared the diets. Raanan PG40 commercial diet (370 g kg<sup>-1</sup> crude protein and 95 g kg<sup>-1</sup> total lipid) was used as control for the experiment (PG40). Three test diets (MM30, MM50 and MM80) were formulated to be comparable to PG40 (isonitrogenous and isoenergetic) by gradually replacing high-priced imported FM (20; 50 and 70% substitution, respectively) and SBM (10, 20 and 35% substitution, respectively) with locally available MM (30, 50 and 80 g kg<sup>-1</sup>, respectively) and cheaper PBM (80, 100 130 g kg<sup>-1</sup>, respectively). The substitution levels of FM and SBM were driven by the limited quantity of MM available for the experiment. Then, nutritional composition (protein levels) was adjusted by addition of PBM as part as a least-cost strategy. Furthermore, FO was not included in the 3 test diets due to the high lipid content of the MM (244.5 g kg<sup>-1</sup>). Chemical compositions of the diets was analysed as described below (Table 1). Commercially packaged diets were kept on-farm under cool and shaded conditions (25°C, 50-60% relative humidity) and used within two months after manufacture.

[Insert Table 1]

### *Experimental Design*

In order to demonstrate the relevance of the results, the experiment was conducted on-farm (commercial tilapia producer, Volta Lake, Ghana) under conditions similar to commercial husbandry practices. All-male, hormonally sex-reversed Nile tilapia fingerlings (*O. niloticus*) were obtained from a local commercial hatchery. Prior the start of the experiment, twenty-five thousand (25,000) fish were transferred into a single

floating cage (3x3 m) suspended in Volta Lake where they were fed six times a day with a standard diet (480 g kg<sup>-1</sup> crude protein and 50 g kg<sup>-1</sup> total lipid) for 3 weeks as an acclimation period to the lake conditions. Twelve floating cages (1 m<sup>2</sup> cage<sup>-1</sup>), set up in the outermost part of the grow-out and nursery site of the farm (500 m from the shore, water column of 30-35 m depth), were then stocked at random with one thousand five hundred (1,500) acclimated fingerlings (5.7±0.5 g fish<sup>-1</sup>) each. The experiment was conducted between the months of September and October 2014, for 32 days which was equivalent to the commercial advanced nursing period and allowed a body increase of at least 300% recommended for juvenile fish studies (NRC, 2011). Control and test diets were distributed daily by hand to triplicates cages; fish were fed to visual satiety, over 6 feeding sessions day<sup>-1</sup> (at regular intervals of 2 hours) and amount of feed distributed was determined by difference with pre-weighed feed containers prepared daily. Water temperature (°C), pH and dissolved oxygen (DO; mg L<sup>-1</sup>) were recorded daily at 07:00 hrs and 16:00 hrs using OxyGuard® Handy digital probes (Polaris and pH) immersed at 50 cm under the water surface within cages.

At the start and on termination of the experiment, all the fish in each cage were counted and bulk weighed (Tanita KD 200 digital scale, precision: 0-1000gx1g). Growth was monitored through intermediate samplings carried out every 10 days, by counting and recording bulk weights of 3 separate sub-samples from each cage (representing approximately 20% of the population), using a scoop net of fish concentrated in the corner of the cage. Fish were starved for 24 hours prior samplings in order to limit stress and mortalities related to handling. Whole fish samples were collected at the start (n=20 fish from initial population) and on termination (n=5 fish cage<sup>-1</sup>) of the experiment, following

an overdose of metacaine sulfonate (MS-222) anaesthetic. Samples were systematically pooled and homogenized (on a cage basis) and stored at -20°C until further analyses.

#### *Chemical analyses*

MM, diets and whole fish samples were analysed at the University of Stirling (Stirling, UK) using standard methods to determine dry matter by drying at 110°C until constant weight (AOAC 1990); ash content by combustion in a muffle furnace at 600°C (AOAC 1990); crude protein using the Kjeldahl method (calculated as  $N \times 6.25$ ; Persson 2008); crude fibre using Foss FiberCap 2021/2023 system (Foss Application Note ASN3801; Foss Analytical, Hillerød, Denmark) and energy by bomb calorimetry (Gallenkamp autobomb, calibrated with benzoic acid). Lipid content in MM and diets was determined by Soxhlet extraction (Soxtec auto extraction unit; Foss Analytical, Hillerød, Denmark; Christie 2003) following acid hydrolysis (Tecator Soxtec method, Foss Analytical, Hillerød, Denmark). Total lipid from samples was extracted according to Folch *et al.* (1957) and determined gravimetrically. Fatty Acid Methyl Esters (FAME) were then prepared from total lipid by acid-catalysed transesterification (Christie 1993). Extraction and purification of FAME were performed as described by Tocher & Harvie (1988) and separated and quantified by gas-liquid chromatography using a Fisons GC-8160 (Thermo Scientific, Milan, Italy) equipped with a 30 m x 0.32 mm i.d. x 0.25 µm ZB-Wax column (Phenomenex, Cheshire, UK), 'on column' injection and flame ionisation detection. Hydrogen was used as carrier gas with initial oven thermal gradient of 50°C to 150°C at 40°C.min<sup>-1</sup> to a final temperature of 230°C at 2°C.min<sup>-1</sup>. Individual FAME were identified by comparison to known standards (Supelco™ 37-FAME mix; Sigma-Aldrich Ltd., Poole, UK) and published data (Tocher and Harvie, 1988). Data were collected and processed using Chromcard for Windows (Version 1.19; Thermoquest Italia S.p.A.,

Milan, Italy). Fatty acid content ( $\text{g kg}^{-1}$  of sample) was calculated using heptadecanoic acid (17:0) as internal standard. Amino acid composition of the MM and diets ( $\text{g kg}^{-1}$  of sample) was determined by ALS Food and Pharmaceutical (Chatteris, UK) using HPLC method.

#### *Calculations and statistical methods*

Fish performance and feed utilisation were evaluated according the following indices:

- Live Weight Gain (WG, g) = Final live weight ( $W_f$ , g) – Initial live weight ( $W_i$ , g)
- Specific Growth Rate (SGR, %  $\text{day}^{-1}$ ) =  $[\text{Ln}(W_f) - \text{Ln}(W_i)] / \text{days} \times 100$
- Food Conversion Ratio (FCR) = Feed distributed (g DM) / WG
- Daily feeding rate (% biomass  $\text{day}^{-1}$ ) =  $[(\text{Feed distributed (g DM)} / \text{number of feeding days}) / \text{Biomass (g)}] \times 100$
- Protein Efficiency Ratio (PER) = WG / Total protein fed (g DM)
- Survival Rate (%) =  $[(\text{Fish stocked initially} - \text{Mortality}) / \text{Fish stocked initially}] \times 100$

Statistical analyses were carried out using IBM SPSS Statistics software (version 21). Data were subjected to one-way analysis of variance (ANOVA) followed by Tukey's HSD test for unplanned multiple comparisons. Correlations between the dietary inclusions of MM + PBM and the performance or nutritional results were analysed using Pearson's coefficient. A significance of  $P < 0.05$  was considered for all analyses performed.

## **Results**

Water temperature and dissolved oxygen varied slightly during the course of the experiment and the diurnal periods with values ranging from 26.8 to 30.5°C and 5.1 to 8.1  $\text{g L}^{-1}$ , respectively. All the values were within tolerance limits for tilapia (Beveridge



& McAndrew 2000; El-Sayed 2006). Growth and feed utilisation of the fish fed the control and experimental diets were not affected by the treatments (Table 2). During the 32-day experimental period, fish grew from an initial weight of  $5.7 \pm 0.5$  g fish<sup>-1</sup> to a final weight  $16.6 \pm 0.5$  g fish<sup>-1</sup>. Live weight gain and SGR of the fish fed the control and the MM-based diets were not significantly different ( $P \geq 0.05$ ). Overall feeding response was good with total amounts of feed distributed ( $26.0 \pm 0.3$  kg cage<sup>-1</sup>) and feeding rates ( $4.2 \pm 0.2$  % biomass day<sup>-1</sup>) not significantly different between treatments ( $P \geq 0.05$ ), indicating similar feed intake. Feed utilisation efficiency (FCR and PER) was not compromised by the dietary treatments ( $P \geq 0.05$ ). However, MM30 diet led to a significantly lower survival ( $81.7 \pm 1.9$  %) compared to others ( $P < 0.05$ ) and MM80 survival rate ( $90.1 \pm 0.5$  %) was found significantly higher than PG40 ( $86.1 \pm 0.3$  %).

[Insert Table 2]

Analysed fish body compositions compared between treatments indicated no significant differences ( $P \geq 0.05$ ) for dry matter, crude protein, crude lipid, ash and crude fibre (Table 3). However, whole carcass FA composition varied significantly between dietary treatments. Strong linear relationships were found between the dietary inclusion of MM and selected FA; in particular, MM dietary inclusion was positively correlated to the total saturated FA ( $r$  0.672;  $P < 0.05$ ) and a negatively to the n-3 PUFA ( $r$  -0.725;  $P < 0.05$ ).

[Insert Table 3]

## **Discussion**

Nowadays, feed formulation strategies account for the feed ingredients nutritional quality but also cost, availability and sustainability. Local constraints identified in Ghana (cost of importation, poor quality of local materials, etc.) increase the pressure on feed

manufacturers to find cost-efficient alternative feed ingredients to replace always more costly feedstuffs such as marine ingredients (Gabriel *et al.* 2007; Obirikorang *et al.* 2015). According to recent publications (Barroso *et al.* 2014; Henry *et al.* 2015), insect meals from dipterans such as the common housefly (*M. domestica*) or the black soldier fly (*H. illucens*) feature nutritional characteristics similar to FM suggesting that they could be suitable substitutes for this conventional feed ingredient. Nevertheless, compared to high-quality FM such as anchovies, herring or menhaden (NRC, 2011), the BSF meal produced in Ghana for the purpose of the experiment, displayed a lower protein content and higher lipid content. Similar observation was made by Barroso *et al.* (2014) for a BSF meal which presented even lower levels of crude protein, lipid and ash (362 g kg<sup>-1</sup>; 180 g kg<sup>-1</sup> and 93 g kg<sup>-1</sup>, respectively) compared to the BSF meal produced in Ghana. Insect life stage, feeding substrate and processing methods influence their nutritional composition (Aniebo & Owen 2010; van Huis *et al.* 2013) which explains the differences reported here. Also similar to that previously found by Barroso *et al.* (2014), the amino acid profile of the MM was comparable to conventional FM including 9 out of 10 of the essential amino acids (BSF meal is known to be low in tryptophan; Newton *et al.* 1977; Henry *et al.* 2015). It is therefore a great source of protein. MM used in the current experiment was also a rich source of FA, in particular saturated and monounsaturated and it was slightly richer in EPA and DHA compared to MM used in other studies (St-Hilaire *et al.* 2007b; Kroeckel *et al.* 2012; Barroso *et al.* 2014) owing to the substrate mix on which the larvae fed (St-Hilaire *et al.* 2007a). Although MM is locally available (Devic *et al.* 2013) and a suitable source of nutrients for fish as demonstrated in several other studies (St-Hilaire *et al.* 2007b; Kroeckel *et al.* 2012, Lock *et al.* 2015), inclusion in a complete diet in substitution of conventional ingredients such as FM, FO and SBM requires adjustments

in the formulation. Lower protein content of the MM was therefore corrected by increasing the PBM inclusion in the test diets, which is considered as a good source of protein although lacking of some essential amino acids (NRC 2011). In addition, according to El-Sayed (1998), PBM can replace totally FM in practical diets for tilapia and that inclusion up to 470 g kg<sup>-1</sup> did not compromise the fish performance. Total substitution of FO related to the high lipid content of the MM resulted in MM30 and MM50 dietary lipid contents being about 18% lower than PG40 and MM80. Low-fat diets are preferred for warmwater omnivorous fish such as tilapia (El-Sayed 2006) and recommended dietary lipid content for tilapia fingerlings vary between 80 and 120 g kg<sup>-1</sup> (Jauncey 1998). Greater inclusion of MM in such a formulation would have certainly led to lipid contents exceeding the recommended range. Nevertheless, a possible solution could be to use defatted MM instead of crude, which would enable higher inclusion levels as suggested by Fasakin *et al.* (2003). The FA composition of the MM-based diets was also affected by the substitution of FM and FO, nonetheless, essential FA requirements for optimal growth of tilapia fingerlings (C<sub>18</sub> PUFA such as 18:2n-6 and 18:3n-3) were satisfied (NRC 2011). Finally the formulation strategy applied in the current allowed the production of comparable diets in terms of macronutrients and meeting the requirements for tilapia fingerlings (Jauncey 1998; El-Sayed 2006; NRC 2011).

Fish performance were acceptable for tilapia farmed in cages (El-Sayed 2013) and not significantly different among treatments, indicating that dietary treatments did not compromise the growth. This result is comparable to those of other studies where MM or PBM were used as alternative ingredients in practical diets for tilapia in substitution of FM (Ogunji *et al.* 2008a, b; El-Sayed 2013; Sing *et al.* 2014). Also, similar to that previously reported in other studies (Fasakin *et al.* 2003; Ogunji *et al.* 2008a;

Karapanagiotidis *et al.* 2014; Sing *et al.* 2014), overall survival was good during the 32-day experimental period. Significant differences in survival were more likely explained by the stress related to frequent sampling and handling which would have more deeply affected the smaller fish (Bolivar *et al.* 2004; MacNiven & Little 2001); indeed, although initial weights were not significantly different between treatments, the fish stocked at a slightly smaller size had significantly lower survival rates than the larger fish. In addition, in comparison with other treatments, the significantly lower survival of MM30-fed fish could explain the slightly (but not significant) better weight gain and SGR ( $11.8 \pm 1.9$  g fish<sup>-1</sup> and  $3.7 \pm 0.4$  % day<sup>-1</sup>, respectively) probably owing to a reduction of the competition for the resources.

The feeding method applied in the experiment (manual distribution), which is common practice in countries where labor costs are low, limits feed wastage and prevents starvation as it is based on the fish feeding response (El-Sayed 2013). Multiple feeding can also improve growth and feed efficiency in species such as tilapia with relatively small stomachs and a continuous foraging behaviour (Shiau 2002; NRC 2011). Feed utilisation efficiency, measured through feeding rates, FCR and PER, was comparable between treatments. Feed intake was not affected by the MM dietary inclusions and the retroactively calculated feeding rates indicated that the fish were appropriately fed. Indeed, at 28°C, it is recommended to feed 5 to 20 g tilapia fingerlings at 6-4 % biomass day<sup>-1</sup> (Shiau 2002; Ng & Romano 2013). Palatability of feeds containing insect meal seems to be related to various factors such as the fish species and its feeding response but also the insect meal characteristics (species, farming and processing methods) (Henry *et al.* 2015). For instance, a diet containing defatted BSF meal seemed to be poorly palatable for juvenile turbot, *Psetta maxima* (Kroeckel *et al.* 2012), whereas inclusion of blowfly

meal in juvenile red tilapia feed did not affect the feed intake (Sing *et al.* 2014). Consistent with other studies (Ogunji *et al.* 2008; Sing *et al.* 2014), PER values were comparable between dietary treatments indicating that dietary proteins were similarly and efficiently used by the fish fed the different diets (Steffens 1989; De Silva & Anderson 1994). The proximate composition of the fish was also not affected by the dietary treatments. However, the FA profile of the fish carcasses mirrored that of the diets and strong correlations indicated that dietary inclusions of MM, in particular its FA composition, influenced the FA composition of the whole fish body. The total substitution of the FO in the 3 experimental diets explained the n-6 and n-3 PUFA levels (respectively increasing and decreasing with increasing MM inclusions). Sánchez-Muros *et al.* (2015) made similar observations while replacing 50 % FM and 100 % FO with a *Tenebrio molitor* larvae meal in a diet for Nile tilapia fingerlings. At the juvenile stages, the farmers prioritize optimal growth and survival, using cost-effective and sustainable feeds and ingredients, and FA composition of the fish carcass is less concerning than for a market-size fish (Turchini *et al.* 2009). To restore the n-3 PUFA levels, which have beneficial effects on human health (Ruxton *et al.* 2004), finishing diets containing essential PUFA could be used during the last weeks of farming (fattening stage), improving, therefore, the nutritional quality of the marketable size fish (Karapanagiotidis *et al.* 2007). Commercial aquafeed manufacturers continue to produce feeds for tilapia including 20 to 250 g kg<sup>-1</sup> FM due to its high nutritional quality (FAO 2012). In the current study, the absence of differences between the fish growth performance, feed utilisation and body composition under the different dietary treatments lead to the conclusion that inclusions of up to 80 g kg<sup>-1</sup> MM and 130 g kg<sup>-1</sup> PBM, substituting FO totally, up to 70% FM and 35% SBM do not affect the feed quality for advanced nursing tilapia. Providing that the

300 market price of the MM is competitive, feed production costs would be alleviated by the  
301 reduction of FM, FO and SBM and the strategic use of quality ingredients such as MM  
302 and PBM to balance the diet. More broadly, inclusions of cheaper, sustainable and locally  
303 available feedstuffs in juvenile tilapia commercial feed could support the sustainable  
304 intensification of aquaculture and contribute more widely to food security.  
305

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311

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479

480 **Tables**

481 **Table 1** Ingredient composition (g kg<sup>-1</sup>) of the control (PG40) and the 3 test diets (MM30,  
 482 MM50 and MM80) and proximate, amino acid, and fatty acid compositions (g kg<sup>-1</sup> of  
 483 meal) of the Black Soldier Fly (BSF) larvae meal and the diets. Values are presented ‘as  
 484 is’, based on duplicate analyses.

		Experimental diets			
		PG40	MM30	MM50	MM80
<b>Ingredient composition (g kg<sup>-1</sup>)</b>					
Fish meal	-	100.0	80.0	50.0	30.0
Soybean meal	-	200.0	180.0	160.0	130.0
BSF meal	-	-	30.0	50.0	80.0
Poultry byproduct meal	-	50.0	80.0	100.0	130.0
Fish oil	-	20.0	-	-	-
Corn meal	-	304.0	304.0	304.0	304.0
Wheat bran	-	130.0	130.0	140.0	130.0
Poultry blood meal	-	100.0	100.0	100.0	100.0
Feather meal	-	90.0	90.0	90.0	90.0
Vitamin premix	-	3.0	3.0	3.0	3.0
Anti-mold	-	1.5	1.5	1.5	1.5
Klinofeed ®	-	1.0	1.0	1.0	1.0
Methionine	-	0.5	0.5	0.5	0.5
<b>Proximate composition (g kg<sup>-1</sup>)</b>					
Dry matter	950.3	949.4	957.5	952.5	958.3
Crude protein	416.4	372.8	378.4	371.7	376.7
Crude lipid	232.4	94.8	78.3	77.6	93.4
Crude fibre	76.6	30.5	33.1	35.0	34.4
Ash	116.5	62.9	67.6	68.1	66.8
Nitrogen-Free Extract	108.4	388.4	400.1	400.1	387
Gross Energy (MJ/kg)	21.7	19.6	19.2	19.4	19.7
<b>Essential amino acids (g kg<sup>-1</sup>)</b>					
Arginine	20	13.6	13.7	13.1	13.3
Histidine	11.8	22.5	23.7	23.4	24.1
Lysine	27	21.8	21.8	21.5	21.8
Methionine	7.5	11.2	11.7	11.4	11.5
Phenylalanine	17.5	16.2	15.7	16.3	16.6
Leucine	29	6.6	5.8	5.1	5.9
Iso-Leucine	18.4	21.7	21.8	22.4	22.2
Valine	26.3	19.3	19.6	19.6	19
Threonine	17.2	34.1	34.3	34.4	34.4



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**Fatty acid composition (g kg<sup>-1</sup>)**

14:00	10.2	1	1	1.3	1.6
16:00	33.3	14.4	9.7	10.3	10.7
18:00	4.7	3.6	3.2	3.5	3.6
20:00	0.1	0.3	0.2	0.2	0.2
Total saturated <sup>1</sup>	49.2	19.7	14.4	15.6	16.5
16:1n-7	6	1.4	1.2	1.3	1.4
18:1n-9	26.7	24.1	16.9	17.1	16.9
18:1n-7	5.5	1.5	1.3	1.3	1.4
22:1n-11	0.3	0.7	0.4	0.3	0.3
Total monounsaturated <sup>2</sup>	40.8	29.2	20.9	20.9	20.9
18:2n-6	18.6	15.1	12.9	13.3	12.5
20:2n-6	0.3	0.3	0.2	0.2	0.1
20:4n-6	0.2	0.2	0.2	0.1	0.1
Total n-6 <sup>3</sup>	19.2	15.8	13.4	13.7	12.9
18:3n-3	1.7	1.7	1.3	1.2	1.1
18:4n-3	1.9	0.2	0.2	0.1	0.1
20:5n-3 (EPA)	0.9	0.8	0.5	0.4	0.4
22:6n-3 (DHA)	0.1	1.8	1.2	0.8	0.7
Total n-3 <sup>4</sup>	4.6	5.1	3.5	2.7	2.5
Total Polyunsaturated <sup>5</sup>	23.8	21.1	17.1	16.6	15.5
Total fatty acids	113.9	70	52.5	53.1	52.9
n-3/n-6	0.2	0.3	0.3	0.2	0.2

<sup>1</sup>Includes 15:0; 22:0 and 24:0 ; <sup>2</sup>Includes 16:1n-9; 20:1n-11; 20:1n-7; 22:1n-9 and 24:1n-9 ; <sup>3</sup>Includes 18:3n-6; 20:3n-6 and 22:4n-6 ; <sup>4</sup>Includes 20:3n-3; 20:4n-3 and 22:5n-3 ; <sup>5</sup>Includes 16:2 and 16:3

**Table 2** Growth performance and feed utilisation indices determined for nursing tilapia fingerlings fed control and experimental diets for 32 days

	Dietary treatments			
	PG40	MM30	MM50	MM80
Initial live weight (g fish <sup>-1</sup> )	5.5±0.2	5.1±0.2	6.1±0.7	6.1±0.3
Final live weight (g fish <sup>-1</sup> )	16.0±0.8	16.9±1.8	17.0±1.1	16.5±0.9
Live weight gain (g fish <sup>-1</sup> )	10.4±0.9	11.8±1.9	10.9±1.5	10.4±0.6
SGR (% day <sup>-1</sup> )	3.3±0.2	3.7±0.4	3.2±0.5	3.1±0.1
Total feed distributed (kg cage <sup>-1</sup> )	25.9±0.9	25.7±0.4	26.2±0.2	26.4±0.6
FCR	2.2±0.1	2.1±0.3	2.0±0.2	2.1±0.1
PER	1.2±0.1	1.2±0.2	1.3±0.1	1.2±0.0
Feeding rate (% biomass day <sup>-1</sup> )	4.4±0.1	4.3±0.4	4.0±0.1	4.1±0.1
Survival rate (%)	86.1±0.3 <sup>b</sup>	81.7±1.9 <sup>c</sup>	89.5±2.2 <sup>ab</sup>	90.1±0.5 <sup>a</sup>

Means± SD (n=3) bearing different superscripts within each row are significantly different (P<0.05)

492 **Table 3** Proximate composition (g kg<sup>-1</sup> of fish, wet weight basis) and fatty acid  
 493 composition (g kg<sup>-1</sup> of fish) of Nile tilapia fingerlings whole body at the start (Initial;  
 494 mean±SD; n=4) and on termination of the 32-day experimental period

	Initial	Dietary treatment			
		PG40	MM30	MM50	MM80
Proximate composition (g kg <sup>-1</sup> )					
Dry matter	238.1±3.4	286.0±5.1	278.5±2.5	282.0±2.5	285.2±1.1
Crude protein	148.8±1.5	153.6±3.0	152.7±1.3	152.9±0.9	154.3±0.5
Crude lipid	37.0±1.4	107.8±6.1	96.1±1.1	99.9±4.4	102.2±6.1
Ash	48.8±0.9	33.1±1.7	34.5±0.8	33.9±2.3	35.7±0.7
Crude fibre	0.7±0.2	0.8±0.1	0.8±0.1	0.8±0.1	0.8±0.1
Fatty acid composition (g kg <sup>-1</sup> fish)					
14:0	0.50±0.03	1.57±0.09 <sup>c</sup>	1.70±0.13 <sup>c</sup>	2.32±0.21 <sup>b</sup>	2.91±0.25 <sup>a</sup>
16:0	4.99±0.20	15.92±0.93 <sup>ab</sup>	14.57±0.57 <sup>b</sup>	16.65±1.99 <sup>ab</sup>	18.01±1.03 <sup>a</sup>
18:0	1.83±0.07	4.80±0.33	4.68±0.11	4.96±0.52	5.21±0.36
20:0	0.08±0.00	0.18±0.00	0.17±0.00	0.18±0.03	0.19±0.01
<i>Total saturated</i> <sup>1</sup>	7.56±0.30	22.68±1.35 <sup>ab</sup>	21.34±0.84 <sup>b</sup>	24.35±2.78 <sup>ab</sup>	26.57±1.64 <sup>a</sup>
16:1n-7	0.93±0.05	2.71±0.16 <sup>b</sup>	2.58±0.12 <sup>b</sup>	2.94±0.37 <sup>ab</sup>	3.32±0.12 <sup>a</sup>
18:1n-9	7.04±0.33	25.42±1.83	22.11±0.80	24.72±2.94	25.70±1.38
18:1n-7	0.84±0.04	2.04±0.13 <sup>ab</sup>	1.96±0.06 <sup>b</sup>	2.28±0.32 <sup>ab</sup>	2.52±0.20 <sup>a</sup>
22:1n-11	0.08±0.00	0.36±0.02 <sup>a</sup>	0.18±0.02 <sup>b</sup>	0.20±0.02 <sup>b</sup>	0.18±0.01 <sup>b</sup>
<i>Total monounsats.</i> <sup>2</sup>	9.72±0.46	32.98±2.28	28.92±0.97	32.55±3.89	34.26±1.80
18:2n-6	2.54±0.19	8.16±0.52	7.61±0.20	8.21±1.05	9.08±0.52
20:2n-6	0.22±0.01	0.61±0.05	0.58±0.01	0.63±0.07	0.67±0.03
20:4n-6	0.35±0.02	0.60±0.05 <sup>b</sup>	0.60±0.04 <sup>b</sup>	0.66±0.08 <sup>ab</sup>	0.76±0.05 <sup>a</sup>

<b>Total n-6<sup>3</sup></b>	3.69±0.27	10.91±0.77	10.24±0.36	11.10±1.44	12.33±0.72
18:3n-3	0.21±0.02	0.74±0.05	0.62±0.03	0.62±0.09	0.66±0.04
20:4n-3	0.03±0.00	0.11±0.01 <sup>a</sup>	0.07±0.00 <sup>b</sup>	0.07±0.01 <sup>b</sup>	0.07±0.00 <sup>b</sup>
20:5n-3 (EPA)	0.07±0.00	0.13±0.02 <sup>a</sup>	0.08±0.01 <sup>b</sup>	0.08±0.01 <sup>b</sup>	0.09±0.01 <sup>b</sup>
22:5n-3	0.16±0.01	0.47±0.04 <sup>a</sup>	0.32±0.03 <sup>b</sup>	0.29±0.04 <sup>b</sup>	0.32±0.02 <sup>b</sup>
22:6n-3 (DHA)	0.89±0.03	1.85±0.19 <sup>a</sup>	1.38±0.12 <sup>b</sup>	1.16±0.17 <sup>b</sup>	1.24±0.05 <sup>b</sup>
<b>Total n-3<sup>4</sup></b>	1.43±0.06	3.55±0.32 <sup>a</sup>	2.66±0.18 <sup>b</sup>	2.38±0.35 <sup>b</sup>	2.56±0.12 <sup>b</sup>
<b>Total polyunsat.<sup>5</sup></b>	5.30±0.34	14.77±1.09	13.19±0.53	13.79±1.82	15.21±0.82
<b>Total fatty acids</b>	22.58±1.08	70.42±4.71	63.46±2.17	70.68±8.42	76.04±4.22

Mean± SD (n=3) bearing different superscripts within each row are significantly different (P<0.05); comparisons were made between dietary treatments and excluded the initial values.

<sup>1</sup>Includes 15:0; 22:0 and 24:0

<sup>2</sup>Includes 16:1n-9; 17:1; 20:1n-11; 20:1n-9; 20:1n-7; 22:1n-9 and 24:1n-9

<sup>3</sup>Includes 18:3n-6; 20:3n-6; 22:4n-6 and 22:5n-6

<sup>4</sup>Includes 18:4n-3 and 20:3n-3

<sup>5</sup>Includes 16:2; 16:3 and 16:4